J. Clin. Chem. Clin. Biochem.
Vol. 20, 1982, pp. 731–734

Insulin Binding to Erythrocytes After Acute 16-Methyleneprednisolone Ingestion

By *A. Dwenger, W. Holle, R. Zick* and *I. Trautschold*

Abteilung für Klinische Biochemie des Zentrums Biochemie und Arbeitsbereich Diabetologie des Zentrums Innere Medizin, Medizinische Hochschule Hannover

(Received February 15/May 28, 1982)

Summary: The binding of [^{125}I]insulin to erythrocytes, glucose and insulin were determined before and 1, 7 and 35 days after ingestion of 2 × 60 mg 16-methyleneprednisolone. None of two groups of volunteers (7 males, 4 females) showed clear alterations of the insulin binding parameters (K_a and R_0), or of the fasting cortisol, glucose and insulin concentrations.

These results exclude the possibility that the diabetogenic effect of glucocorticoids is accompanied by an alteration of the insulin receptor characteristics of erythrocytes.

Insulinbindung an Erythrocyten nach akuter Gabe von 16-Methylen-prednisolon

Zusammenfassung: Die Bindung von [^{125}I]Insulin an Erythrocyten sowie die Konzentrationen von Cortisol, Glucose und Insulin im Plasma wurden vor und 1, 7 und 35 Tage nach Gabe von 2 × 60 mg 16-Methylen-prednisolon ermittelt. In keinem Falle (7 Männer, 4 Frauen) konnten deutliche Änderungen der Insulin-Bindungsparameter sowie der Nüchtern-Cortisol-, -Glucose- und -Insulin-Konzentrationen beobachtet werden.

Die vorliegenden Ergebnisse schließen aus, daß die diabetogene Wirkung von Glucocorticosteroiden an einer Änderung der Characteristika der Insulin-Rezeptoren von Erythrocyten erkannt werden kann.

Introduction

Glucocorticoid administration is known to produce insulin resistance (1) and it has been suggested that the diabetogenic effect of corticosteroids might be mediated, at least in part, via changes in insulin receptors (2). Until now quite different results on the effect of glucocorticoids on insulin binding have been reported (2, 3, 5, 9). Therefore insulin binding on erythrocytes was studied in normal volunteers after an acute high-dose steroid treatment which is currently being used clinically in man.

Materials and Methods

Subjects

Two groups of subjects were investigated:

(A) 7 healthy males and

(B) 4 healthy females.

Each of them received each 60 mg of 16-methyleneprednisolone (Decortilen, Merck) at two successive days. Heparinized blood samples were drawn by venipuncture in the fasting state before steroid ingestion as well as 24 hours after the second dosage, 7 days later and after 5 weeks.

Insulin binding studies

Insulin binding was performed according to the procedure of *Gambhir* (6) with slight modifications (4). Isolated erythrocytes ($3.5–4.5 \cdot 10^{12}$/l) were incubated with [^{125}I]insulin (0.05 nmol/l) and additionally with unlabeled insulin (0.09–174 nmol/l) at 15 °C for 3 hours; nonspecific binding was determined in the range between 0.5 and 17.4 µmol/l of unlabeled hormone and an average was subtracted from total binding to give specific insulin binding.

Analytical procedures

Insulin (7) and cortisol (Amersham/Buchler) were determined radioimmunologically, glucose was measured by the glucose oxidase method (Boehringer/Mannheim).

0340-076X/82/0020-0731$02.00

Calculation procedures

In view of our recent finding of a critical ligand concentration (8), the analysis of binding data was performed on the initial part of the *Scatchard* plot up to a total ligand concentration of about 2 nmol/l. Individual *Scatchard* plots were made for each subject, and receptor affinity and concentration were calculated from the initial points of the *Scatchard* plot by linear regression methods. Statistical analyses were carried out using the *Wilcoxon* test for paired data. From the total binding data, the analytical, the intra- and the interindividual variances were calculated.

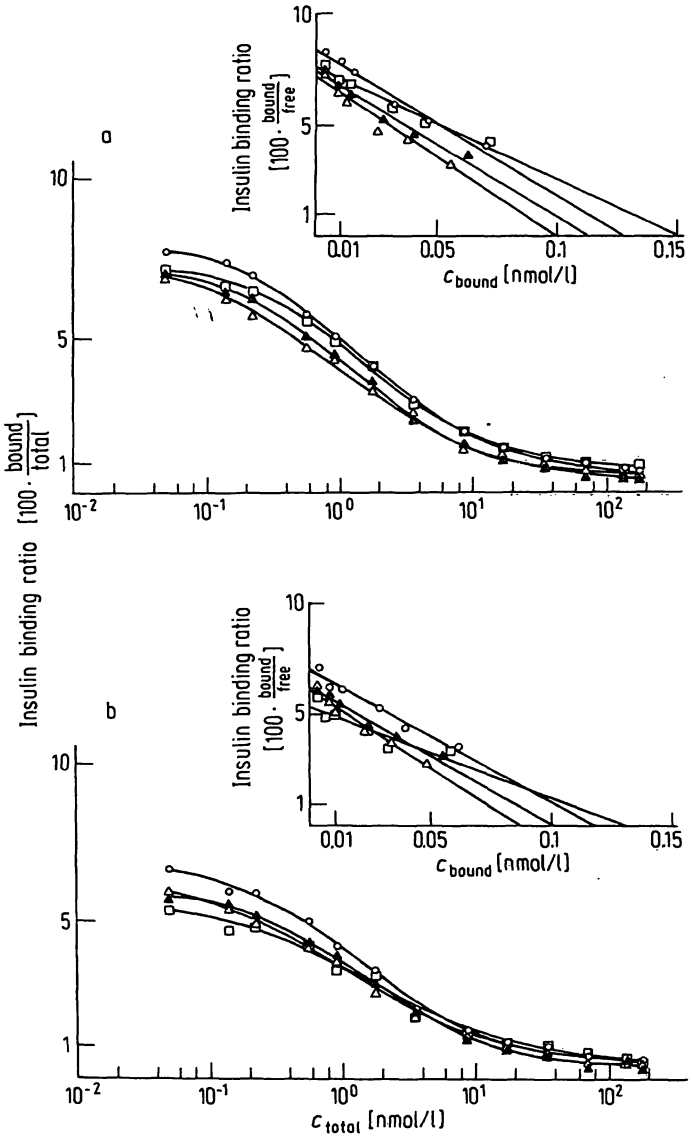

Fig. 1. Specific insulin binding to human erythrocytes ($4 \cdot 10^{12}$/l) before (▲) as well as 1 day (○), 7 days (□) and 35 days (△) after administration of 2 × 60 mg 16-methyleneprednisolone.
[^{125}I]insulin binding ($\frac{B}{T}$) in dependence on the total insulin concentration (nmol/l).
a = means of the data from 7 males
b = means of the data from 4 females
Insets: *Scatchard* plots derived from the corresponding binding data; slopes of the straight lines (linear regression up to 2 nmol/l) indicate receptor affinity; intercepts with the abscissa indicate the maximal amount of insulin bound.

Results

After an acute glucocorticoid treatment no significant changes of the erythrocyte insulin receptor affinity and concentration could be observed 1, 7 and 35 days after ingestion of the steroid in males (fig. 1 and 2, tab. 1).

Endogenous cortisol tended to decrease one day after steroid administration and to increase afterwards whereas insulin seemed to be slightly elevated after one day followed by a normalization, but none of the differences were significant. Plasma glucose levels, too, did not exhibit significant changes (fig. 3). In females there were slight differences in K_a between days 7 and 35 as well as in R_0 between days 1 and 35 and between days 7 and 35. But these differences could be calculated only for $p < 0.2$ (fig. 2 and tab. 1). Plasma cortisol, insulin and glucose concentrations did not change significantly in females (fig. 3).

Tab. 1. Significance of differences in erythrocyte insulin receptor affinity (K_a) and concentration (R_0) before (0) and after (1, 7, 35 days) administration of 16-methyleneprednisolone calculated by *Wilcoxon*'s test for paired data.

A	n = 7		Males	$p < 0.05$			
K_a	35	7	1	R_0	35	7	1
0	ns	ns	ns	0	ns	ns	ns
1	ns	ns		1	ns	ns	
7	ns			7	ns		

B	n = 4		Females	$p < 0.2$			
K_a	35	7	1	R_0	35	7	1
0	ns	ns	ns	0	ns	ns	ns
1	ns	ns		1	s	ns	
7	s			7	s		

Analysis of variances was performed for the lower (0.05–2 nmol/l) and the higher (17.4–174 nmol/l) ranges of total ligand concentrations separately. The analytical variances were 4–10% CV for the lower range, 19–44% CV for the higher range; the corresponding data for the intraindividual variances were 12–21% and 37–56%, and for the interindividual variances 45–47% and 58–65% CV.

Discussion

By employment of a modified *Scatchard* analysis (8), the current results show no modulation of the erythrocyte insulin receptor after 16-methyleneprednisolone administration. This is in agreement with the results of *Fantus* et al. (9) who did not find any alteration on monocyte insulin receptors after prednisone ingestion. In contrast, *Yasuda* et al. (5) reported a decrease of the receptor affinity on erythrocytes and *Beck-Nielsen* et al. (3) found an increase of the receptor concentration on monocytes. These contradictary results can be partially explained by the individual data handling of each investigator (4). There are slight differences of the experimental procedures, like the size of the corticosteroid doses and the time of blood withdrawal after steroid ingestion. But these should influence the degree rather than the quality of alterations of the receptor.

Receptor binding data were calculated from the terminal part of the *Scatchard* plot. As can be seen by the analysis of variances one would expect sufficient reliability of the results only from data in the lower range of total ligand concentrations, i.e. from the initial part of the *Scatchard* plot. We demonstrated that the binding parameters K_a and R_0 calculated from the initial part of the *Scatchard* plot (up to total ligand concentrations representing the half maximal inhibition concentration of the system) were nearly identical with those calculated by employment of a non-linear least squares method (8). Therefore we focused our calculation on the initial part of the *Scatchard* plot.

Our results exclude the possibility that the diabetogenic effect of glucocorticosteroids is reflected in alterations of the erythrocyte insulin receptor characteristics.

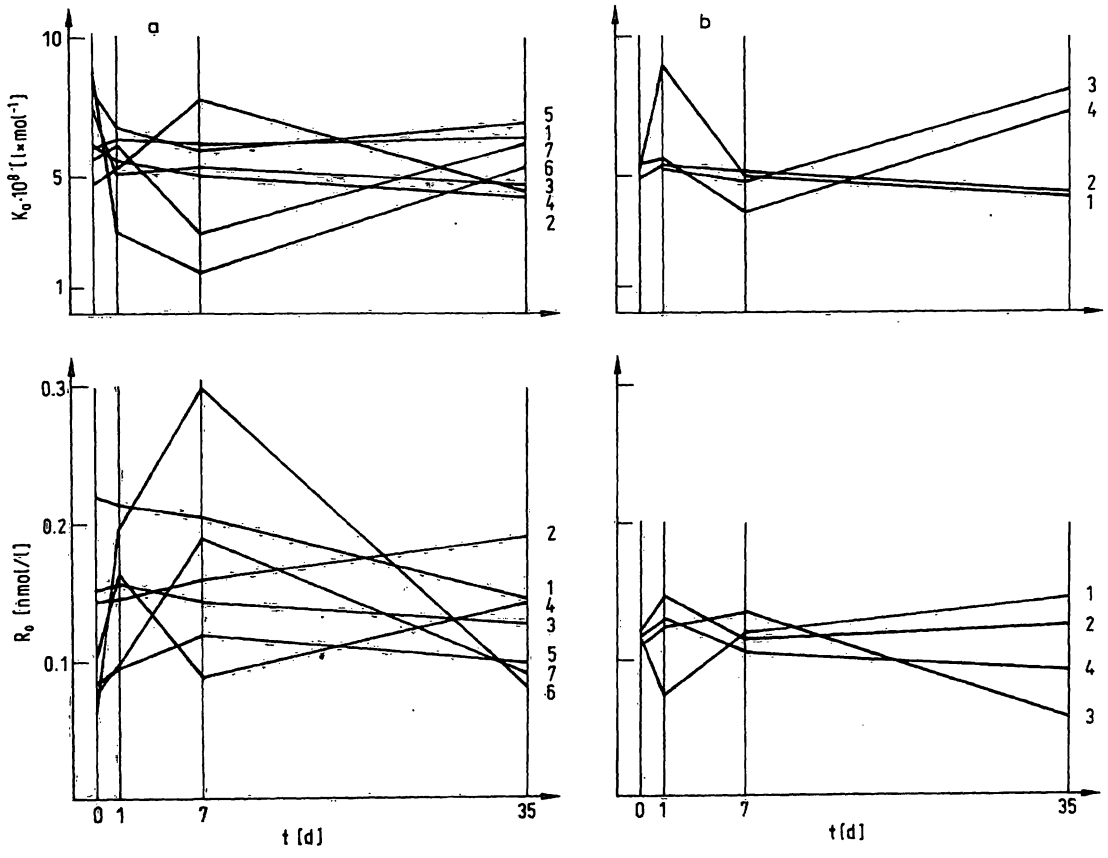

Fig. 2. Affinity (K_a) and concentration (R_0) of the erythrocyte insulin receptors before (0) and after (1, 7, 35 days) administration of 2 X 60 mg 16-methyleneprednisolone for 7 males (a) and for 4 females (b).

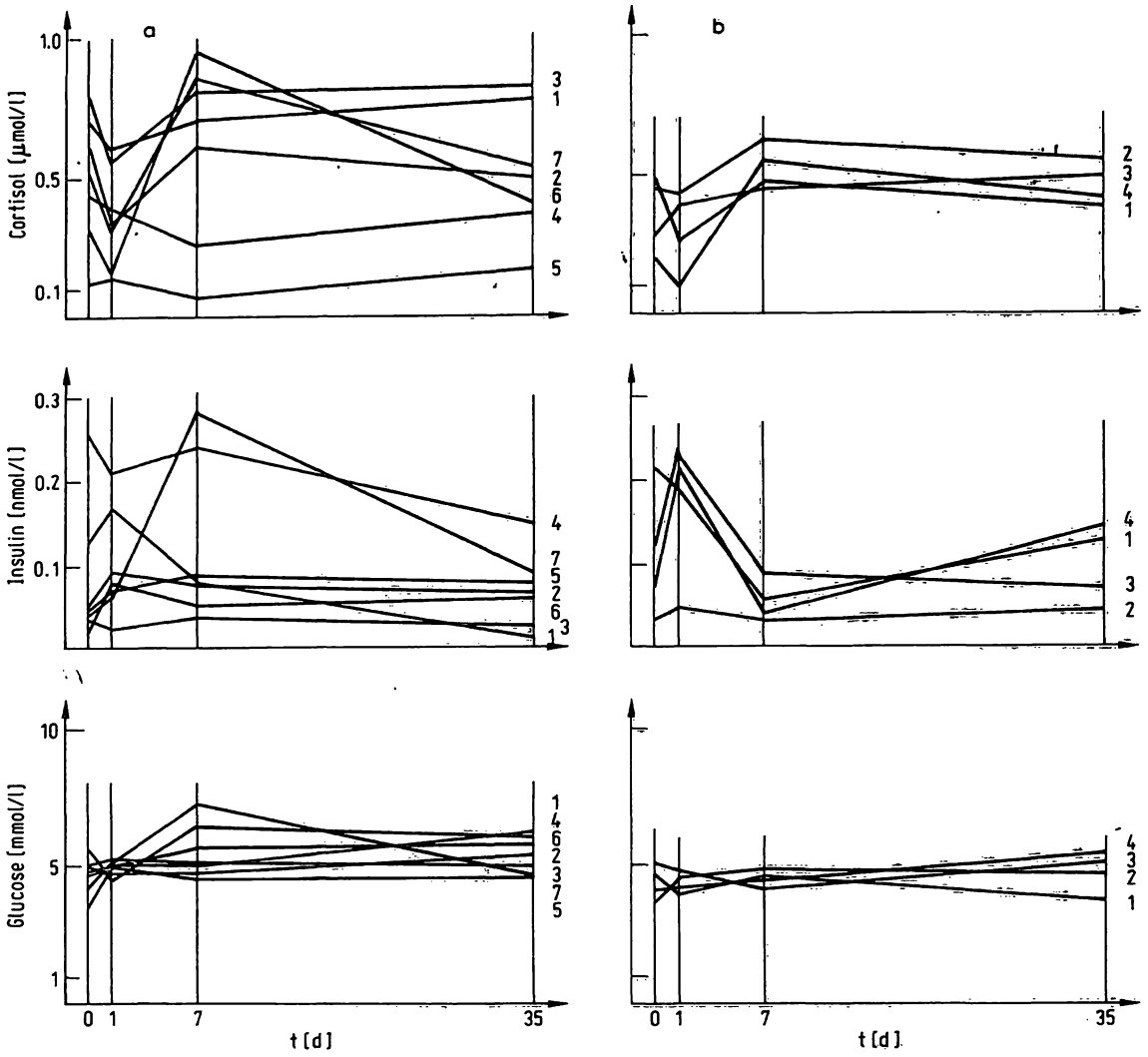

Fig. 3. Plasma cortisol, insulin and glucose concentrations before (0) and after (1, 7, 35 days) administration of 2 × 60 mg 16-methyleneprednisolone for 7 males (a) and for 4 females (b).

References

1. Munck, A. (1971) Perspect. Biol. Med. *14*, 265–289.
2. Olefsky, J. M., Johnson, J., Liu, F., Jen, Ph. & Reaven, M. (1975) Metabolism *24*, 517–526.
3. Beck-Nielsen, H., De Pirro, R. & Pedersen, O. (1980) J. Clin. Endocrinol. Metab. *50*, 1–4.
4. Dwenger, A., Mitzkat, H.-J., Holle, W., Tost, P. & Trautschold, I. (1982) J. Clin. Chem. Clin. Biochem. *20*, 273–279.
5. Yasuda, K. & Kitabchi, A. E. (1980) Diabetes *29*, 811–814.
6. Gambhir, K. K., Archer, J. A. & Bradley, C. J. (1978) Diabetes *27*, 701–708.
7. Dwenger, A., Tost, P. & Trautschold, I. (1977) J. Clin. Chem. Clin. Biochem. *15*, 593–602.
8. Dwenger, A., Tost, P. & Trautschold, I. (1982) Fresenius' Z. Anal. Chem. *311*, 471–472.
9. Fantus, G., Ryan, J., Hizuka, N. & Gorden, P. (1981) J. Clin. Endocrinol. Metab. *52*, 953–960.

Dr. rer. nat. Alex Dwenger
Abteilung für Klinische Biochemie
Zentrum Biochemie
Medizinische Hochschule Hannover
Karl-Wiechert-Allee 9
D-3000 Hannover 61